

Comparison of Five Poxvirus Genomes by Analysis with Restriction Endonucleases *Hind*III, *Bam*I and *Eco*RI

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(Accepted 1 August 1977)

SUMMARY

The DNAs of rabbitpox, vaccinia, cowpox, ectromelia and fowl pox virus, all grown in the same host system, were cleaved with restriction endonucleases *Hind*III, *Bam*I and *Eco*RI. The resulting digestion products were separated by electrophoresis on agarose slab gels. From the similarities and dissimilarities of the migration profiles obtained, it was concluded that vaccinia and rabbitpox virus are genetically the most closely related viruses investigated, although their DNA cleavage patterns are clearly distinguishable. Cowpox and ectromelia virus both show about the same degree of relatedness to each other as to rabbitpox and vaccinia virus. Fowl pox virus, which belongs to the genus *Avipoxvirus*, contains the largest genome examined (approx. 160×10^6 daltons) and the cleavage patterns of its DNA show no similarities with those of orthopoxvirus DNAs, thus indicating a very low degree of genetic relatedness. We believe that restriction analysis is a useful method for the identification and classification of poxvirus isolates.

INTRODUCTION

Poxviruses, the largest known viruses, are widely distributed in the animal kingdom. They are found in mammals, birds and insects. Systematically, they belong to the family Poxviridae (Fenner, 1976) with six genera and more than forty species. Classification is mainly based on phenotypic criteria such as morphology, antigenicity, host range and behaviour in cell cultures (Mayr, Mahnel & Munz, 1972). A newly isolated poxvirus can easily be assigned, e.g. to the genus *Orthopoxvirus* (formerly *variola/vaccinia* subgroup) by making use of the close serological relationship among its members. Identification within the genus however, is more complicated (Mahnel, 1974; Baxby, 1975) and does not always allow an unequivocal classification. For example, it still remains to be determined whether rabbitpox viruses are strains of vaccinia virus or whether they represent a separate species. Apart from this particular question, the isolation of a number of poxviruses sharing properties with smallpox viruses revealed limitations in the traditional methods for poxvirus identification. Therefore, Baxby (1975), emphasized the need for new techniques and approaches that are not dependent on the response of secondary systems to infection.

While much work has been done on the biochemistry of vaccinia virus, direct comparisons of biochemical or physico-chemical properties of members of the poxvirus family are rare. An attempt was made by Bellet & Fenner (1968), who determined nucleic acid sequence homologies using DNA-RNA hybridization techniques.

Bacterial restriction endonucleases provide a further way to characterize genomes, but

Dissertation von Heinz K. Müller
unter der Leitung von
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1978

whereas the DNAs of small viruses, such as simian virus 40 (Danna & Nathans, 1971), papilloma virus (Favre *et al.* 1975), phage lambda (Allet *et al.* 1973) and others have been extensively studied, the large molecular size of the poxvirus genomes (and hence the large number of fragments resulting from endonuclease cleavage of entire genomes) seemed to preclude such an analysis. Indeed, digestion of vaccinia virus DNA (DeFilippes, 1976) and other poxvirus genomes in our laboratory with either *Hpa*I, *Hpa*II or *Hap*II yields more than 50 fragments. Only recently have Gangemi & Sharp (1976), Parr, Burnett & Garon (1976), and Müller *et al.* (1977) shown that, using selected enzymes, these large genomes can also be cleaved into a set of readily analysable fragments.

In this communication we compare the DNA cleavage patterns of five different poxviruses obtained by digestion with three different restriction endonucleases. The phenotypic relatedness between these poxviruses is well reflected by the similarities and dissimilarities of the migration profiles after electrophoresis of the digestion products.

Using the mathematical approach described in the accompanying paper (Schümperli, Lagadec & Müller, 1978) we have tried to estimate the degree of sequence homology between the four orthopoxviruses under investigation.

METHODS

Poxviridae. The following representatives were analysed: (Nomenclature according to Mayr *et al.* 1972; Fenner, 1976) *Orthopoxvirus commune* (vaccinia virus), strain Elstree; *Orthopoxvirus bovis* (cowpox virus), strain Brighton (red pox); *Orthopoxvirus muris* (ectromelia virus), isolate E-5/1973, Munich; *Orthopoxvirus cuniculi* (rabbitpox virus), strain Utrecht; *Avipoxvirus gallinae* (fowl pox virus), isolate HP-1, Munich.

Virus growth and purification. All viruses were grown on the chorioallantoic membrane of developing chick-embryos and purified by the method of Joklik (1962), except that, after banding in sucrose gradients, the virions were not dialysed, but twice resuspended in 1 mM-tris-HCl (pH 9.0) and pelleted by centrifugation at 30000 g for 30 min.

DNA isolation. DNA was isolated using method 2-2 of Sarov & Becker (1967) except that the digestion buffer was made up of standard saline citrate (1 × SSC = 0.15 M-NaCl, 0.015 M-Na-citrate) and the Pronase B (Calbiochem) had been pre-treated as described by Kates & Beeson (1970). Briefly, up to 100 E_{260} /ml of virions (Joklik, 1962) were incubated overnight at 37 °C in a solution of 1 × SSC, 27% sucrose, 1% sodium-deoxycholate and 200 µg/ml of Pronase and the DNA was extracted with phenol-chloroform (1:1) saturated with 1 × SSC. After exhaustive dialysis against 50 mM-tris-HCl (pH 7.8), 1 mM-EDTA, the DNA was concentrated to about 0.1 µg/µl, using a modification of the polyethyleneglycol procedure described by Kohn (1959). A drop of chloroform was then added to the preparation and the DNA solution was stored at 4 °C.

Restriction of DNA. Digestion of DNAs with *Hind*III restriction endonuclease (Smith & Wilcox, 1970) was carried out in 50 mM-tris-HCl (pH 7.8), 7 mM-MgCl₂, containing 0.1 volume of a saturated aqueous solution of para-hydroxymercuribenzoate. *Eco*RI digestion was done in 100 mM-tris-HCl (pH 7.3), 10 mM-MgCl₂. For *Bam*I experiments the buffer consisted of 10 mM-tris-HCl (pH 8.0), 10 mM-MgCl₂, 50 mM-NaCl and 14 mM-β-mercaptoethanol. Incubation was for 6 h at 37 °C for *Hind*III and *Eco*RI and at 30 °C for *Bam*I experiments. The reaction was stopped by addition of an equal volume of 'agarose-suspension' (Schaffner *et al.* 1976) which also markedly reduces edge effects in gel electrophoresis.

Gel electrophoresis. After heating the samples for 5 min to 60 °C and quick-cooling in an



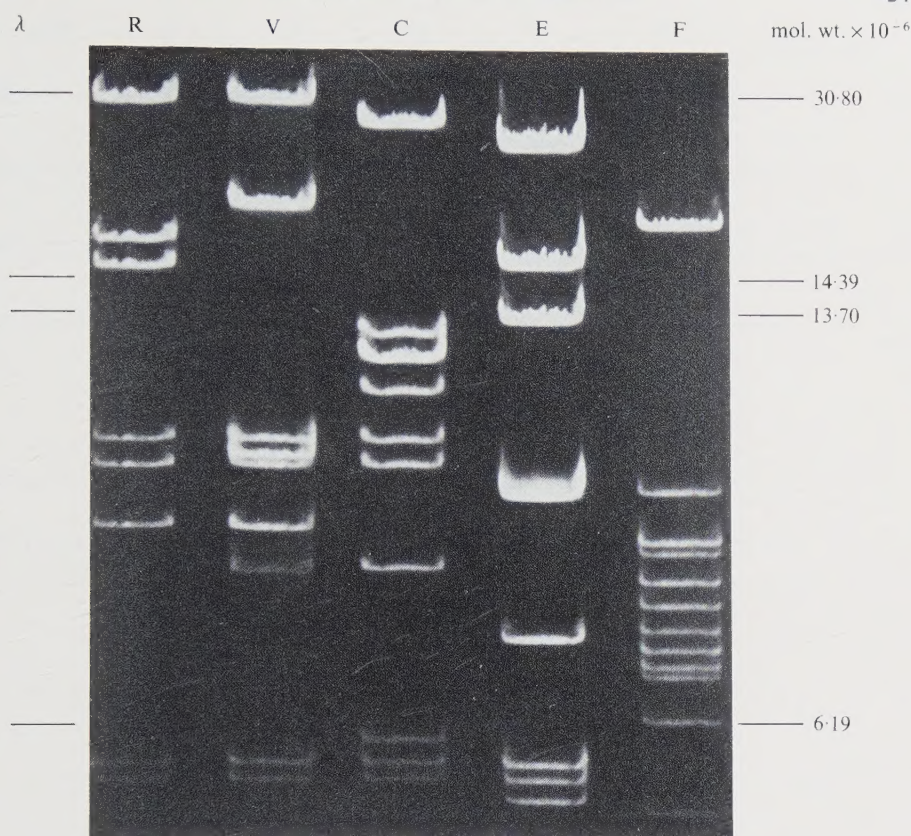


Fig. 1. Patterns obtained after electrophoretic separation of cleavage fragments produced by digestion of rabbitpox (R), vaccinia (V), cowpox (C), ectromelia (E) and fowl pox (F) virus DNA with *Hind*III restriction endonuclease. To resolve 'large' fragments, electrophoresis was on a 0.6% agarose gel for 24 h at 2.5 V/cm. Bars indicate positions of lambda phage (λ) DNA size markers.

ice-bath, they were applied to 0.6% and 1.2% agarose-slab gels ($17 \times 17 \times 0.5$ cm). Lambda phage DNA, either intact or digested with *Eco*RI or *Hind*III was run in parallel as a size marker. Electrophoresis was in tris-phosphate buffer (Loening, 1969) without SDS at 2.5 V/cm for 9 to 12 h for 'small' fragments and for 16 to 24 h for 'large' fragments. After electrophoresis, the gel was stained for 30 min with $1 \mu\text{g/ml}$ ethidium bromide in 50 mM-tris-HCl (pH 7.8), 1 mM-EDTA, placed under a u.v.-lamp and photographed through a red filter with Ilford FP-4 negative film (10.2×12.7 cm).

Evaluation of photographs. For comparison of migration profiles, the negatives were enlarged, the number of bands determined, and the migration distances measured. The molecular weights of the poxvirus DNA cleavage products were determined by comparison to phage lambda DNA fragments of known size (Murray & Murray, 1975; Thomas & Davis, 1975).

RESULTS

In preliminary experiments, the approximate total number of restriction fragments for every enzyme and every DNA was estimated. The fragments were arbitrarily divided into two groups subsequently called 'large' and 'small' fragments. Gel concentrations and

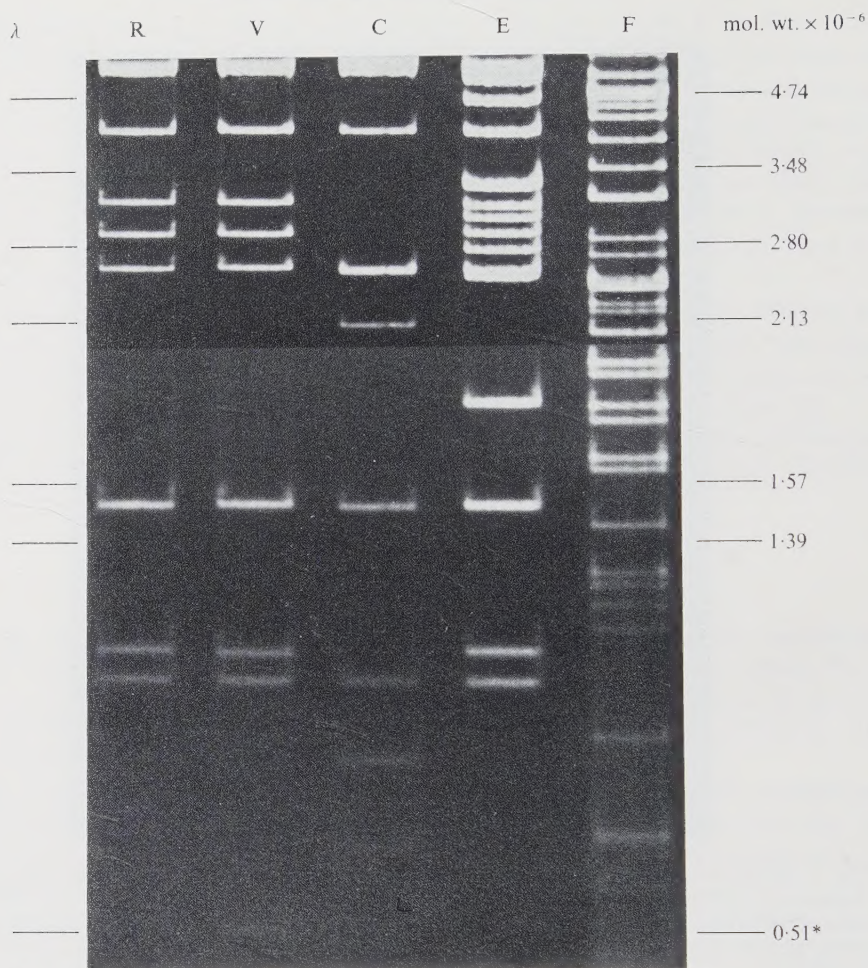


Fig. 2. Patterns obtained after electrophoretic separation of cleavage fragments produced by digestion of rabbitpox (R), vaccinia (V), cowpox (C), ectromelia (E) and fowl pox (F) virus DNA with *Hind*III restriction endonuclease. To resolve 'small' fragments, electrophoresis was on a 1.2% agarose gel for 12 h at 2.5 V/cm. Bars indicate positions of lambda phage (λ) DNA size markers. The asterisk (*) indicates the molecular size of the smallest detectable vaccinia *Hind*III DNA fragment. The tone difference within the photograph results from variations of exposure time for better illustration of faint bands (see also Fig. 4 and 6).

running times were adapted so as to give an optimum separation within these groups (see legends to plates). The resulting migration patterns after electrophoresis are shown in Fig. 1 to 6.

The number of cleavage products and their molecular weights are listed in Tables 1 to 3. For each DNA, the fragments were lettered alphabetically in order of decreasing size. The method used here did not allow us to determine the relative molar quantities of the cleavage products. Therefore, fragments occurring in more than equimolar ratios, as suggested by their increased fluorescent intensity after staining, were considered to be twofold and designated '2x'; consequently, the total number of fragments was indicated as, e.g., 31+4.

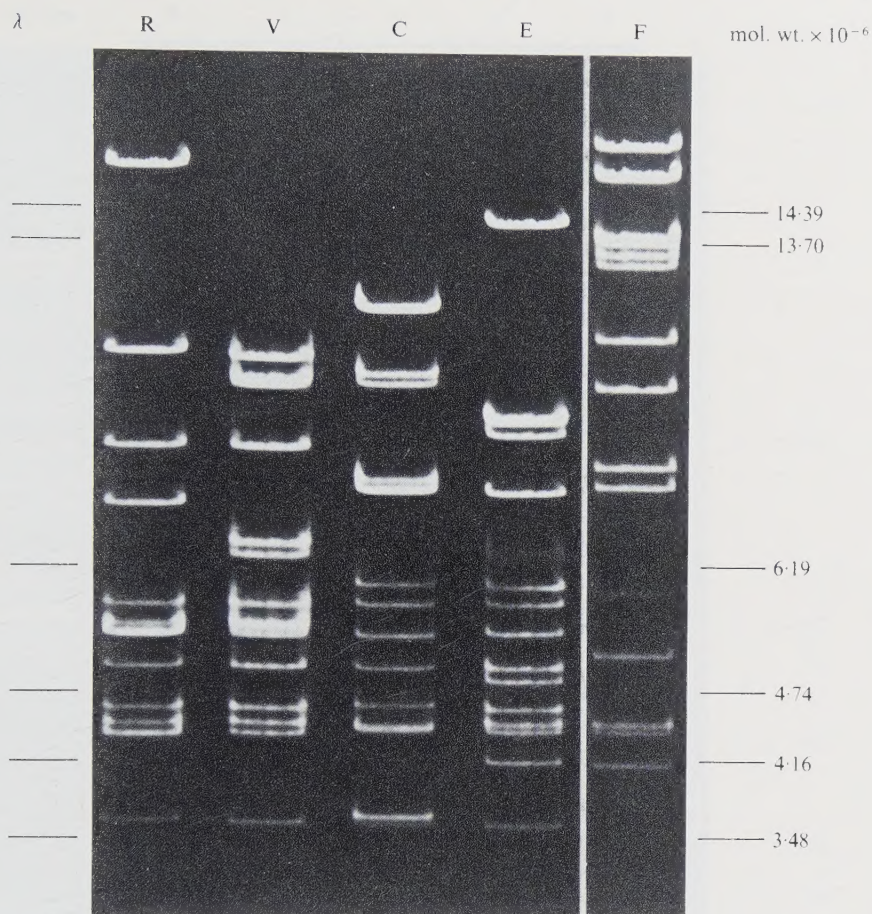


Fig. 3. Patterns obtained after electrophoretic separation of cleavage fragments produced by digestion of rabbitpox (R), vaccinia (V), cowpox (C), ectromelia (E) and fowl pox (F) virus DNA with *Bam*I restriction endonuclease. To resolve 'large' fragments, electrophoresis was on a 0.6% agarose gel for 20 h at 2.5 V/cm. Bars indicate positions of lambda phage (λ) DNA size markers.

DISCUSSION

For this comparison, we have selected four viruses of the genus *Orthopoxvirus* with different degrees of biological relatedness, as well as fowl pox virus which belongs to the genus *Avipoxvirus*. The latter is clearly distinct from the orthopoxviruses with respect to its biological, chemical and physico-chemical characteristics (Randall *et al.* 1964; Gafford & Randall, 1967; White *et al.* 1968; Mayer *et al.* 1972).

The experiments clearly indicate that poxvirus DNAs, although very large in size, can be cleaved into a set of specific fragments which are separable under appropriate electrophoretic conditions. Gangemi & Sharp (1976) reported that their vaccinia DNA was not digested by *Eco*RI and suggested the presence of an enzyme inhibitory substance. All enzymes used in our experiments restricted the DNAs under investigation, *Eco*RI digestions yielding the largest number of fragments.

Co-migration of cleavage products in the gel does not prove their identity, but in the case of closely related DNAs it seems likely that they represent equivalent sections of the genomes

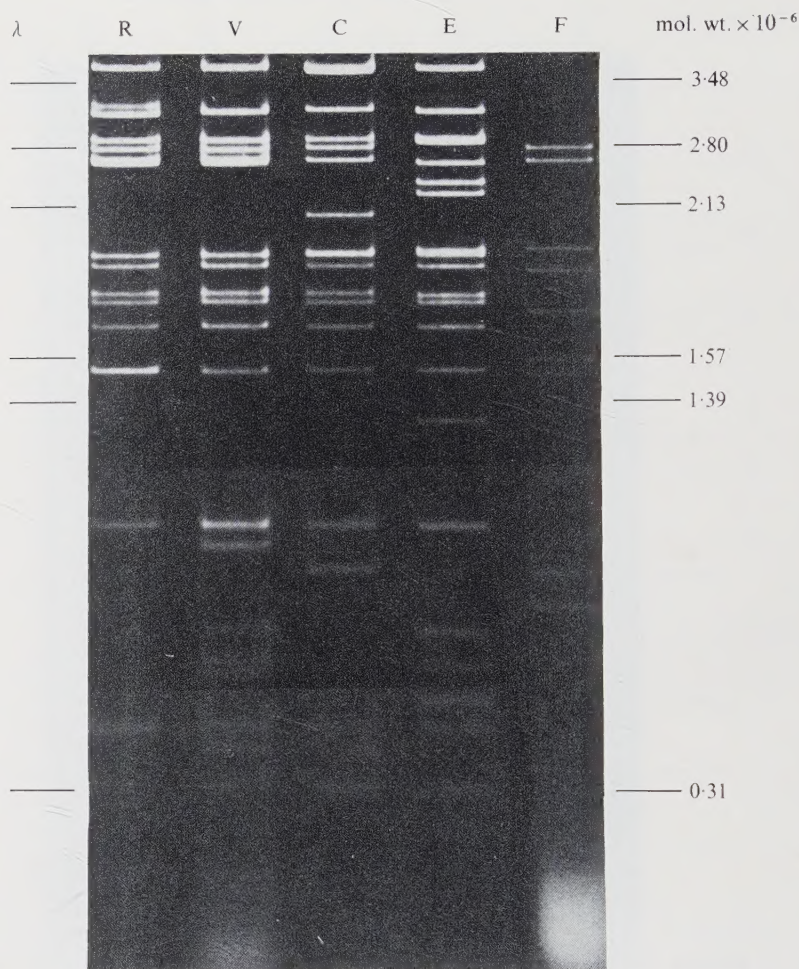


Fig. 4. Patterns obtained after electrophoretic separation of cleavage fragments produced by digestion of rabbitpox (R), vaccinia (V), cowpox (C), ectromelia (E) and fowl pox (F) virus DNA with *Bam*I restriction endonuclease. To resolve 'small' fragments, electrophoresis was on a 1.2% agarose gel for 9 h at 2.5 V/cm. Bars indicate positions of lambda phage (λ) DNA size markers.

compared. Thus, the similarities or dissimilarities of the migration profiles of the different DNAs can be expected to reflect the degree of their genetic relationship.

When, e.g., the *Hind*III patterns (Fig. 1 and 2) are considered it can easily be seen that 12 of the 15 rabbitpox and 17 vaccinia virus DNA fragments, respectively, co-migrate. Cowpox and ectromelia virus DNA yield 18 and 22 fragments of which only 6 are of identical electrophoretic mobility. The cleavage pattern obtained with fowl pox virus DNA shows only minor similarities with those of the orthopoxviruses and with the present data it is difficult to rule out that any co-migration of fragments is not just fortuitous.

Analysis of the gels (shown in Fig. 1 to 6) in this manner leads to the conclusion that, of the four orthopoxviruses examined, rabbitpox and vaccinia virus are very closely related – as is in fact expected from their biological properties – although the cleavage patterns of their DNAs are clearly distinguishable. The migration profiles of cowpox and ectromelia virus DNA are not as similar as those of rabbitpox and vaccinia virus DNA and they have approximately the same number of co-migrating fragments with rabbitpox and vaccinia

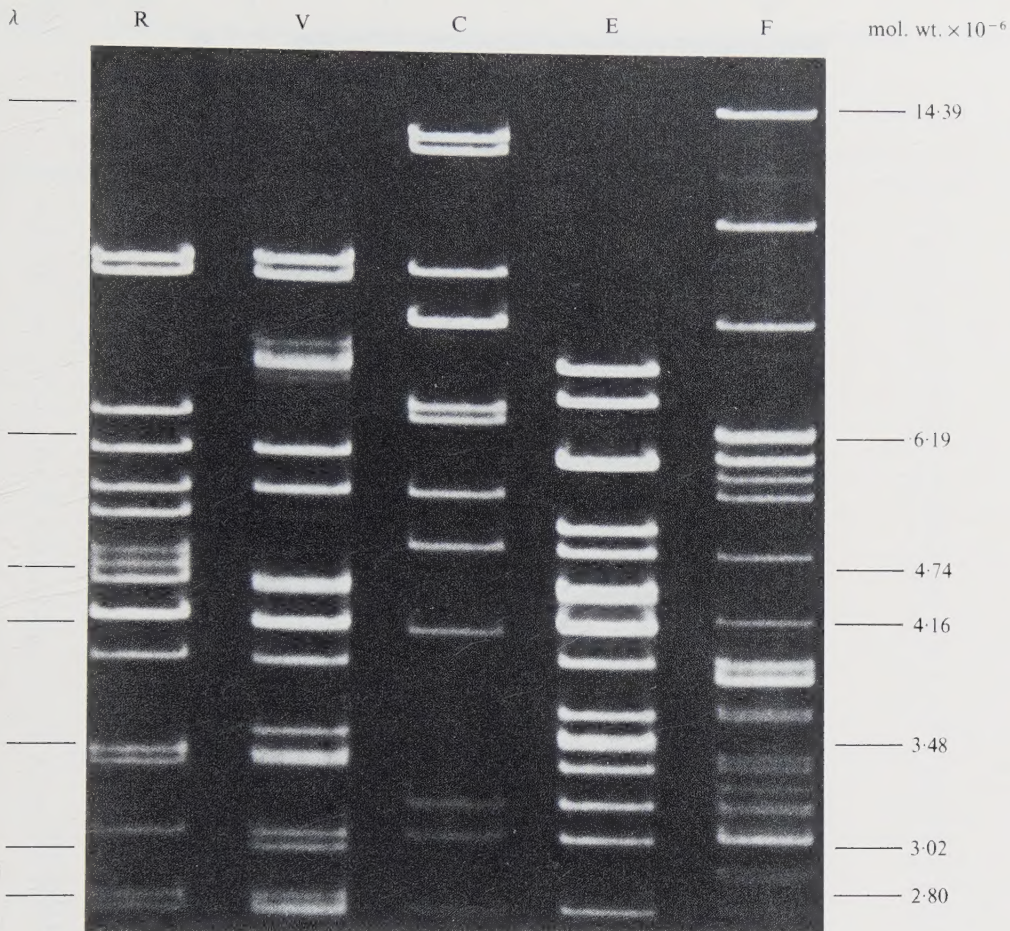


Fig. 5. Patterns obtained after electrophoretic separation of cleavage fragments produced by digestion of rabbitpox (R), vaccinia (V), cowpox (C), ectromelia (E) and fowl pox (F) virus DNA with *Eco*RI restriction endonuclease. To resolve 'large' fragments, electrophoresis was on a 0.6% agarose gel for 16 h at 2.5 V/cm. Bars indicate positions of lambda phage (λ) DNA size markers.

virus DNA as with each other. This would suggest that they are not any more closely related to each other than to rabbitpox and vaccinia virus. This is inconsistent with phenotypic findings (Mahnel, 1974; Baxby, 1975) but is in line with the hybridization data of Bellet & Fenner (1968).

To date, restriction analysis is probably the most sensitive method for comparing closely related genomes, since a single base change in a recognition sequence, undetectable by electron microscopy or by hybridization, leads to a change in the restriction enzyme fragmentation pattern. The chance of recognizing differences in the DNA sequences can be increased by using several suitable restriction enzymes. The limitations in this approach, in that only samples (i.e. the recognition sites) of the total genome are analysed can thereby be reduced.

Although this type of analysis is probably the method of choice to distinguish between very closely related genomes it is not yet possible to decide whether rabbitpox and vaccinia viruses belong to the same species or simply represent different strains. However, a

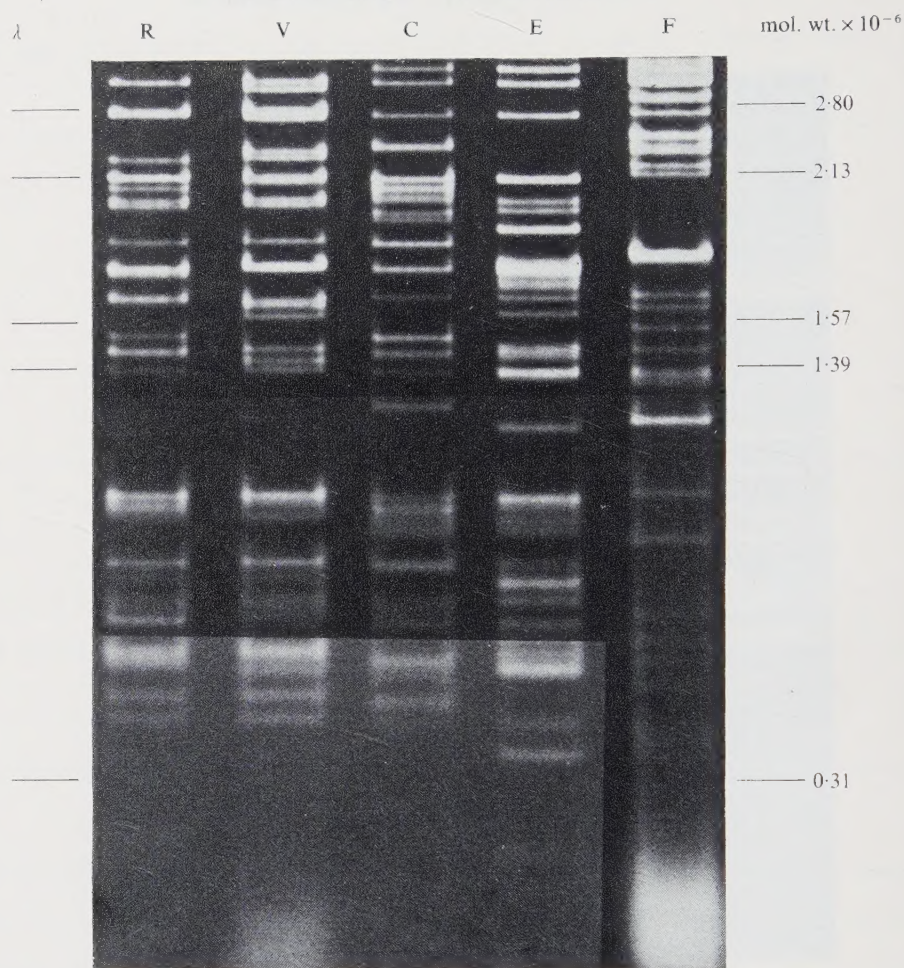


Fig. 6. Patterns obtained after electrophoretic separation of cleavage fragments produced by digestion of rabbitpox (R), vaccinia (V), cowpox (C), ectromelia (E) and fowl pox (F) virus DNA with *EcoRI* restriction endonuclease. To resolve 'small' fragments, electrophoresis was on a 1.2% agarose gel for 9 h at 2.5 V/cm. Bars indicate positions of lambda phage (λ) DNA size markers.

comparison of the *HindIII* restriction patterns of our Elstree strain of vaccinia virus with those published of WR and CV-1 strains (Gangemi & Sharp, 1976) and with some strains from Len Archard (personal communication) revealed only minor differences, suggesting an even closer relatedness among the vaccinia strains.

Summation of the molecular weights of the DNA fragments obtained after complete endonuclease cleavage yields an approximate estimation for the mol. wt. of the total genome. In fact, the size estimates for the different orthopoxvirus DNAs (Tables 1 to 3) are in good agreement with the value determined by Geshelin & Berns (1974) for the vaccinia genome. The mol. wt. estimate for fowl pox virus (approx. 160×10^6) is lower than that reported by Gafford & Randall (1967; 200 to 240×10^6 , 192×10^6) but is, as expected, considerably higher than those of the orthopoxviruses. Interestingly, with all enzymes used, the rabbitpox virus appeared to contain the smallest genome of these viruses, the difference to the

Table 1. *Molecular weight determination of poxvirus DNA fragments produced by cleavage with HindIII endonuclease*

Fragments	Mol. wt. $\times 10^{-6}$ obtained by comparison to λ /RI and λ /HindIII migration distances in agarose gels				
	Rabbitpox	Vaccinia	Cowpox	Ectromelia	Fowl pox
A	29.60	30.00	25.70	22.40	16.00
B	15.95	19.80	13.03	14.81	9.37
C	14.81	10.43	12.50	13.30	8.67
D	10.43	10.15	11.55	9.25	8.47
E	10.00	10.00	10.43	7.12	8.02
F	8.95	8.95	10.00	5.71	7.67
G	5.71	8.20	8.20	5.50	7.27
H	5.50	5.71	5.95	5.35	7.00
I	4.01	5.50	5.71	4.56	6.78
J	3.10	4.01	5.50	4.01	6.68
K	2.85	3.10	4.01	2 $\times$ 3.28	6.21
L	2.54	2.85	2 $\times$ 2.54	3.07	5.29
M	1.51	2.54	2.10	2.98	4.99
N	1.05	1.51	1.51	2.85	4.78
O	0.98	1.05	0.98	2.63	4.74
P		0.98	0.81	2.54	4.47
Q		0.51	0.63	2.45	4.26
R				1.80	3.92
S				1.51	3.46
T				1.05	3.10
U				0.98	2.81
V					2.63
W					2.45
X					2.40
Y					2.30
Z					2.25
AA					2 $\times$ 2.08
AB					1.97
AC					1.95
AD					1.80
AE					1.76
AF					1.66
AG					1.60
AH					1.47
AI					1.29
AJ					1.25
AK					1.18
AL					1.10
AM					0.87
AN					2 $\times$ 0.66
AO					0.58
AP					2 $\times$ 0.45
Fragment total	15	17	17+1	21+1	42+3
Mol. wt.	116.99	125.29	123.69	117.89	170.93

vaccinia DNA being 6 million daltons on average. However, it is clear that the summation of the molecular weights of the fragments can only give an approximate estimation of the total genome size since the relative molar quantities of the migrating fragments cannot unequivocally be determined by the ethidium-bromide method used here, but physical maps of rabbitpox and vaccinia virus DNA which have been established in our laboratory (Wittek *et al.* 1977) show that there is a difference in the molecular size of these two genomes

Table 2. *Molecular weight determination of poxvirus DNA fragments produced by cleavage with BamI endonuclease*

Mol. wt. $\times 10^{-6}$ obtained by comparison to λ /RI and λ /HindIII migration distances in agarose gels					
Fragments	Rabbitpox	Vaccinia	Cowpox	Ectromelia	Fowl pox
A	17.85	9.86	11.19	13.82	21.48
B	10.00	2 $\times$ 9.37	9.50	2 $\times$ 8.75	17.85
C	8.10	8.10	9.37	8.32	13.70
D	7.09	6.48	7.56	7.40	13.24
E	5.69	6.32	2 $\times$ 7.40	5.88	12.80
F	5.47	5.69	5.88	5.69	12.40
G	2 $\times$ 5.36	2 $\times$ 5.47	5.69	5.36	10.52
H	4.99	5.36	5.36	4.99	9.50
I	4.60	4.99	4.99	4.81	7.84
J	4.46	4.60	4.60	4.60	7.56
K	4.40	4.46	2 $\times$ 4.40	4.46	5.08
L	3.76	4.40	2 $\times$ 3.80	4.40	4.50
M	3.11	3.76	3.76	4.17	4.46
N	2.85	3.11	3.11	3.76	4.17
O	2.76	2.85	2.85	3.11	2.76
P	2.64	2.76	2.76	2.85	2.59
Q	2.59	2.64	2.60	2.82	1.92
R	1.89	2.59	2.06	2.59	1.82
S	1.87	1.89	2 $\times$ 1.89	2.33	1.70
T	1.76	1.87	1.87	2.23	1.52
U	1.72	1.76	1.76	1.92	1.45
V	1.64	1.72	1.72	1.89	0.69
W	2 $\times$ 1.45	1.64	1.64	1.87	0.60
X	0.83	1.45	1.45	1.76	< 0.30
Y	0.39	2 $\times$ 0.83	0.83	1.72	
Z	0.31	0.78	0.70	1.64	
AA	< 0.30	0.56	0.39	1.45	
AB	< 0.30	0.50	0.36	1.23	
AC		0.48	0.31	0.83	
AD		0.39	< 0.30	0.56	
AE		0.36	< 0.30	0.48	
AF		0.31		0.41	
AG		< 0.30		0.39	
AH		< 0.30		0.31	
AI		< 0.30		< 0.30	
AJ				< 0.30	
AK					
Fragment total	28 + 2	35 + 3	31 + 4	36 + 1	24
Mol. wt.	114.39	123.02	127.29	127.55	160.15

and that, e.g., the 'heterogeneous' band of vaccinia *HindIII* G (Fig. 1), which could be interpreted as a partial digestion product, represents in fact some heterogeneity in the end-fragment of the molecule (Wittek *et al.* 1977).

Since it was felt that for the determination of genetic relationship from restriction data, quantification of the qualitative information contained in the electrophoresis patterns would be helpful, a mathematical approach to estimate sequence homologies between closely related genomes was developed. This method, described in the accompanying paper (Schümperli *et al.* 1978), yielded upper and lower estimation limits for the genetic relatedness between the orthopoxvirus DNAs (Table 4). Fowl pox virus has not been considered in this approach because its cleavage patterns are so different from the others that the

Table 3. *Molecular weight determination of poxvirus DNA fragments produced by cleavage with EcoRI endonuclease*Mol. wt. $\times 10^{-6}$ obtained by comparison to λ /RI and λ HindIII migration distances in agarose gels

Fragments	Rabbitpox	Vaccinia	Cowpox	Ectromelia	Fowl pox
A	9.50	9.50	13.20	7.40	14.10
B	9.20	9.20	12.90	6.80	10.60
C	6.50	7.50	9.30	2 $\times$ 5.80	8.20
D	6.00	6.00	2 $\times$ 8.20	5.10	2 $\times$ 6.25
E	5.50	5.50	6.70	4.74	2 $\times$ 6.00
F	5.20	2 $\times$ 4.40	6.35	4.40	5.75
G	4.74	2 $\times$ 4.16	5.50	4.33	5.50
H	4.60	3.95	4.80	2 $\times$ 4.16	4.74
I	4.45	3.43	4.16	3.95	4.20
J	2 $\times$ 4.20	3.33	3.15	3.60	2 $\times$ 3.95
K	3.95	3.30	2.95	3.43	2 $\times$ 3.85
L	3.36	3.00	2.68	3.40	3.63
M	3.30	2.90	2.40	3.30	3.60
N	3.00	2.80	2.05	3.15	3.33
O	2.80	2 $\times$ 2.68	2.00	2.95	3.30
P	2.70	2.38	1.99	2.68	3.20
Q	2.30	2.30	1.95	2 $\times$ 2.05	3.15
R	2 $\times$ 2.13	2.13	1.93	1.97	3.10
S	2.05	2.05	1.85	1.95	2 $\times$ 2.95
T	2.00	2.00	1.75	2 $\times$ 1.90	2.86
U	1.97	1.97	1.67	1.75	2.84
V	1.85	1.85	1.50	1.72	2.68
W	1.75	2 $\times$ 1.75	1.45	2 $\times$ 1.69	2.48
X	1.72	2 $\times$ 1.59	1.39	1.67	2.45
Y	1.63	1.58	1.28	1.58	2.35
Z	1.50	1.47	0.83	1.47	2.13
AA	2 $\times$ 1.45	1.40	0.82	1.45	2.05
AB	1.39	1.39	0.76	2 $\times$ 1.37	2 $\times$ 1.78
AC	2 $\times$ 0.83	2 $\times$ 0.83	0.73	1.05	1.67
AD	0.82	0.82	2 $\times$ 0.69	0.83	1.59
AE	0.80	0.80	0.60	0.82	1.52
AF	0.70	2 $\times$ 0.70	0.57	0.76	1.47
AG	0.68	0.68	0.55	0.73	1.37
AH	0.66	0.66	0.49	0.69	1.33
AI	0.60	0.60	0.47	0.67	1.10
AJ	0.57	0.57	2 $\times$ 0.42	0.66	0.83
AK	0.50	0.50	0.37	0.55	0.71
AL	0.49	0.48	< 0.30	0.54	0.57
AM	0.44	0.44	< 0.30	0.52	0.53
AN	0.40	0.40	< 0.30	0.48	0.49
AO	< 0.30			0.46	0.35
AP	< 0.30			0.37	0.33
AQ	< 0.30			0.33	
AR				< 0.30	
AS				< 0.30	
AT				< 0.30	
AU				< 0.30	
Fragment total	43+4	40+7	40+3	47+6	42+6
Mol. wt.	116.84	119.11	119.73	119.62	153.66

Table 4. *Nucleotide sequence homology estimates (%)*

	Vaccinia	Cowpox	Ectromelia
Rabbitpox	86.9-97.1	77.0-95.2	72.7-94.1
Vaccinia	—	75.9-95.1	73.8-94.3
Cowpox	—	—	74.4-94.5

estimation could not be expected to yield reliable results. Although the ranges obtained overlap largely, we believe that the values support the conclusions drawn from the similarities and dissimilarities of the migration profiles.

Restriction analysis of poxvirus DNAs by several suitable endonucleases, such as *Hind*III, *Bam*I, *Sst*I (Wittek *et al.* 1977) and *Kpn*I (A. Menna, personal communication) will certainly prove to be an accurate and useful method in identification and classification of new isolates. Although comparison of the *Sst*I cleavage patterns obtained with rabbitpox virus grown on the chorioallantoic membrane or in HeLa cells were identical we would tend to grow test and reference viruses in the same host system to rule out any possible difference due to the action of host modification enzymes. Since orthopoxviruses are easily grown to high yields and since a variety of restriction enzymes are already commercially available the expense on time and finances lies in a range comparable to that for traditional methods. Assignment to the corresponding taxonomic group could be performed relying on a minimum of subjective criteria, since the fragment pattern obtained after electrophoretic separation of the cleavage products is characteristic of the original DNA molecule in much the same way that the tryptic fingerprint of a protein is characteristic of that particular protein.

We are grateful to Dr D. Baxby (Liverpool), Dr H. Mahnel and Dr A. Mayr (Munich) for kindly providing us with poxviruses, Dr S. Clarkson and Dr C. Weissmann (Zurich) for the generous gift of restriction enzymes and lambda DNA and Susanne Stoffel for skilful technical assistance. We thank Elaine Browse, Dr E. Peterhans and Dr L. Archard for critically reading the manuscript as well as Mrs G. Oss for secretarial help. This work was supported by the State of Zurich and the Swiss National Science Foundation. (H. K. Müller was a participant of the 9th Postgraduate-Course in Experimental Medicine and Biology of the University of Zurich.)

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(Received 19 January 1977)

